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Bee pathogens in *Apis florea* samples accidentally introduced into Malta and Italy

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INTRODUCTION & AIM

Apis florea, the red dwarf honey bee (Figure 1), is a wild bee species native to Asia. Its adaptability and nesting behaviour make it a potential invasive alien species.

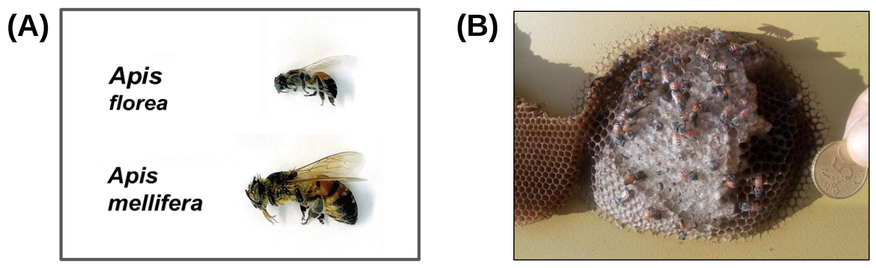

Figure 1. *A. florea* vs *A. mellifera* (A); nest of *A. florea* discovered at the port of Gioia Tauro, Italy (B).

In 2024, after the first detection in Europe of a fully established colony of *A. florea* in Malta (Uzunov *et al*. [1]), a nest was found in the same area near the Malta Freeport Terminals, in the southern part of the island (Figure 2A). In May of the same year, a small nest of *A. florea* was discovered at the port of Gioia Tauro, in the Calabria region (Italy), on the wall of a container coming from India (Figure 2B). This study aims to investigate the presence of known and emerging honey bee pathogens in *A. florea* samples in order to increase the available knowledge on this species and assess the potential introduction of novel pathogens in the local apoidean population.

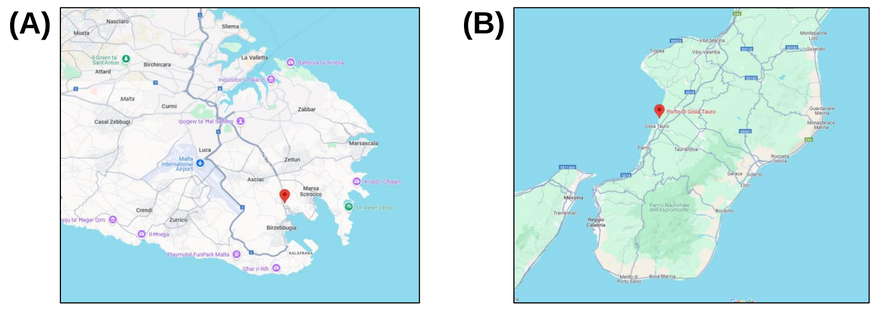

Figure 2. Locations where *A. florea* was found in Malta (A) and Italy (B)

METHODS

From each nest, after morphological identification of the samples, a pool of 25 bees was homogenized. RNA and DNA extraction were performed using the High Pure Viral Nucleic Acid Kit (Roche Diagnostics) and the QIAamp® DNA Mini Kit (Qiagen), respectively. The presence of pathogens was investigated using the methods reported in Table 1. Confirmation of *A. florea* species was performed by sequencing a region of the mitochondrial cytochrome c oxidase subunit I (COI) gene (Folmer *et al*. [2]).

METHODS	PATHOGENS	PRIMER & PROTOCOL REFERENCE
Real time RT-PCR	Acute BeeParalysis Virus (ABPV) Chronic Bee Paralysis virus (CBPV) Deformed Wing Virus (DWV) Sacbrood Virus (SBV) Black Queen Cell Virus (BQCV)	Bordin *et al.* [3]
One step RT-PCR	Kashmir Bee virus (KBV) Israeli Acute Paralysis Virus (IAPV)	Bordin *et al.* [3]
Two step RT-PCR	Lake Sinai Virus (LSV) Rhabdovirus-1 (ARV-1) Rhabdovirus-2 (ARV-2) Bee Macula-like Virus (BMLV) Aphid Lethal Paralysis Virus (ALPV)	Wamonje *et al.* [4] Levin *et al.* [5] Eliash *et al.* [6] deMiranda *et al.* [7] Hou *et al.* [8]
PCR	*Apis Mellifera* Filamentous Virus (AmFV) *Nosema ceranae/Nosema apis* *Lotmaria Passim/Chritidia Mellificae*	Hartmann *et al.* [9] Martín-Hernández *et al.* [10] Bartolomé *et al.* [11]

Table 1. Methods, primers and protocols' references for each pathogen investigated

Amplification products for IAPV, KBV, AmFV, ALPV, ARV-1, ARV-2, BMLV, LSV and the COI gene were analyzed by capillary electrophoresis on LabChip GX Touch HT® (Perkin Elmer). If present, the PCR products were sequenced using Sanger method and the sequences were compared with those available in the GenBank database using BLAST software.

RESULTS

Among all the pathogens investigated, viral coinfections were found only in the sample from Malta; the results are presented in table 2.

	ABPV	CBPV	DWV	SBV	BQCV	KBV	IAPV	AmFV	LSV	ARV1	ARV2	BMLV	ALPV	*N. ceranae*	*N. apis*	*L. passim*	*C. mellificae*
Apis florea Italy				■													
Apis florea Malta	■		■	■	■			■									

Table 2. Pathogens detected (red square) in *A. florea* samples collected in Italy and Malta.

The sequence of the AmFV amplification product (~551 bp) showed 97% identity with the sequences available in the GenBank database (accession number: OR371979.1). Sequencing analysis of COI gene PCR products (~710 bp) confirmed the morphological identification of *A. florea* with an identity of 98.32% and 97.68% for the Italian and Maltese samples, respectively (GenBank accession number: AP018491.1).

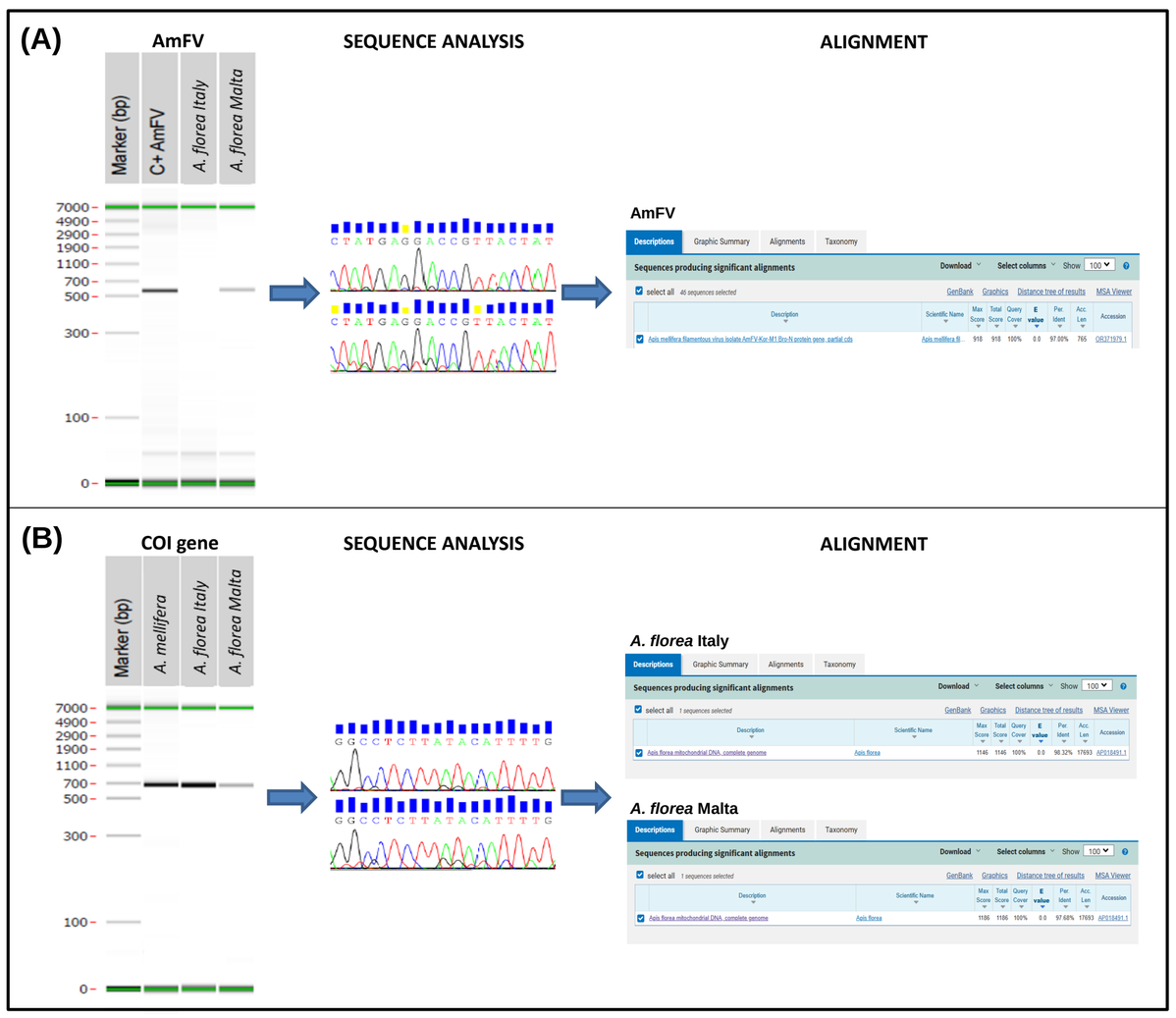

Figure 3. Capillary electrophoresis of the PCR products, sequence analysis and alignment for AmFV (A) and the COI gene (B).

CONCLUSIONS

The spread of *A. florea* in the Mediterranean region, due to global trade, poses an ecological risk to local biodiversity. Furthermore, the presence of pathogens in *A. florea* populations raises concerns about their potential introduction and spread to local honey bee populations, highlighting the need to monitor the potential presence of *A. florea* nests through an early warning system and to take prompt action for their eradication.

REFERENCES

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